ISOLATION OF A CRYSTALLINE ALBUMIN FROM MILK* 485

The relationship between proteins from blood serum and milk has been the subject of considerable study, with some contradictory results (1–5). This is especially true of albumins. Application of electrophoretic techniques to the analysis of whey proteins indicated the presence of a small component, consisting of approximately 3 to 5 per cent of the total protein, with the electrophoretic mobility of a blood serum albumin. In view of the recent isolation, crystallization, and characterization of blood serum albumin (6), it was of interest to crystallize bovine whey albumin and compare its properties with those of crystalline serum albumin.

EXPERIMENTAL

15 gallons of skim milk (approximately 48 liters of whey) were treated by the procedures described for the separation of casein and isolation of crystalline β-lactoglobulin (7). Casein was removed by isoelectric precipitation at pH 4.7. The whey was then separated into two main fractions at ammonium sulfate concentrations of 2.3 and 3.3 M and pH 6.0. The 3.3 M salt fraction was dialyzed to free it of salt, and the crystals of β-lactoglobulin formed at pH 5.3 were removed by centrifugation. The mother liquor, which contained a complex mixture of proteins amounting to about 80 gm. in 7 to 10 liters of salt-free solution, was fractionated further at an alkaline pH.

Step 1, a. Fractionation with Ammonium Sulfate in Presence of Borate— The mother liquor was adjusted to pH 9.1 by the addition of sodium borate to a concentration of 0.05 M, and ammonium sulfate was added to a final concentration of 2.4 M. After the solution had stood at room temperature for a few hours, a sticky, brown-green, peroxidase-containing precipitate floated to the top and was removed (Precipitate 1, 17 gm.). The clear filtrate was brought to 2.6 M with ammonium sulfate, and the precipitate (Precipitate 2, 19 gm.) was removed by filtration. The protein remaining in the filtrate was precipitated completely with 3.4 M ammonium sulfate (Precipitate 3) at pH 5.0.

* Presented at the 117th meeting of the American Chemical Society at Philadelphia, April 9–13, 1950.

† One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

Step 1, b—Precipitate 3 (28 gm.) was dissolved in water to a concentration of about 2 per cent, and separated into three fractions as in Step 1, *a*, but at concentrations of 2.6 (8 gm.), 2.8 (12 gm.), and 3.4 M ammonium sulfate.

Step 2. Alcohol Fractionation—The fraction precipitated with 3.4 M salt (7 gm.) was dissolved in a minimum amount of water and freed of the salt by dialysis. It was then equilibrated by dialysis with 10 per cent alcohol at pH 5.3 and ionic strength 0.005 to 0.01 at 3°. A clear, gelatinous precipitate formed that was removed by centrifugation. The alcohol concentration was then increased to 20 per cent, the precipitate removed by centrifugation, the temperature lowered to −5°, and the alcohol concentration increased to 33 per cent by the slow addition of 95 per cent alcohol to the supernatant. The precipitated protein obtained in this manner (5 gm.) consisted of 47 per cent albumin, as determined by electrophoretic analysis. The supernatant of the 33 per cent alcohol precipitate was adjusted to pH 4.8, ionic strength 0.02, and 40 per cent alcohol concentration at −5°. The precipitate formed was predominantly albumin mixed with three other globulin components (Fig. 1, *c*).

Step 3. Crystallization of Whey Albumin—The globulin components were separated, and the albumin was obtained in crystalline form by further fractionation with ammonium sulfate. The precipitate obtained with 40 per cent alcohol was dissolved in water and the alcohol removed by dialysis. The protein solution was made 0.1 M with dipotassium acid phosphate and 0.03 M with sodium caprylate. Ammonium sulfate was added to a concentration of 2.6 M; the precipitate that formed was removed by filtration, and a saturated ammonium sulfate solution was added until a slight turbidity appeared. After the solution had stood overnight at 3°, both crystals and some amorphous material formed. On warming to room temperature, the crystals dissolved. The amorphous material was removed by filtration, and, after the solution was cooled to 3°, feathery, needle-like crystals again formed (200 mg.). Increasing the salt concentration produced another crop of crystals.

Alternate Crystallization Procedures—The yield was increased considerably by isolating the albumin from the 33 per cent alcohol precipitate. This fraction (5 gm.) consisted of 47 per cent of albumin and 37 per cent of a component with an electrophoretic velocity of 4.4 sq. cm. $volt^{-1}$ $sec.^{-1}$ in veronal buffer at pH 8.4 (Fig. 2). After the alcohol was removed, the dissolved protein solution was made 0.1 M with respect to dipotassium acid phosphate and 0.02 M with sodium caprylate. It was then mixed with an equal volume of saturated ammonium sulfate. The precipitate that formed was essentially the protein with an electrophoretic mobility of 4.4 sq. cm. $volt^{-1}$ $sec.^{-1}$. The protein in the supernatant was completely precipitated

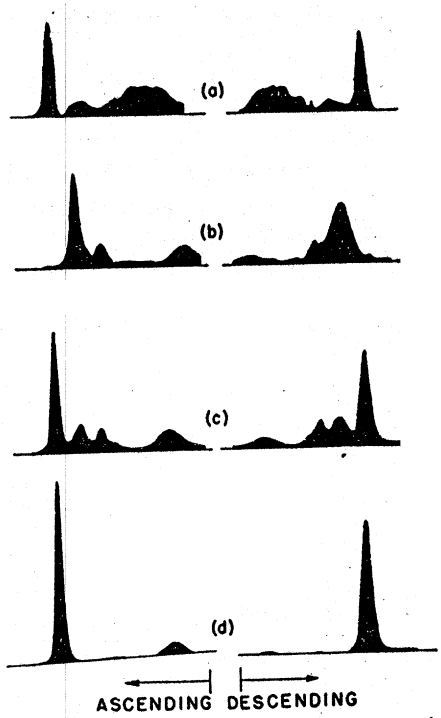

FIG. 1. Electrophoretic patterns in veronal buffer of 0.1 ionic strength (0.05 N sodium veronal, 0.05 N sodium chloride) at pH 8.4 of (*a*) bovine blood serum, (*b*) bovine milk whey, (*c*) whey protein precipitated with 40 per cent alcohol, and (*d*) crystalline bovine whey albumin after electrophoresis for 180 minutes.

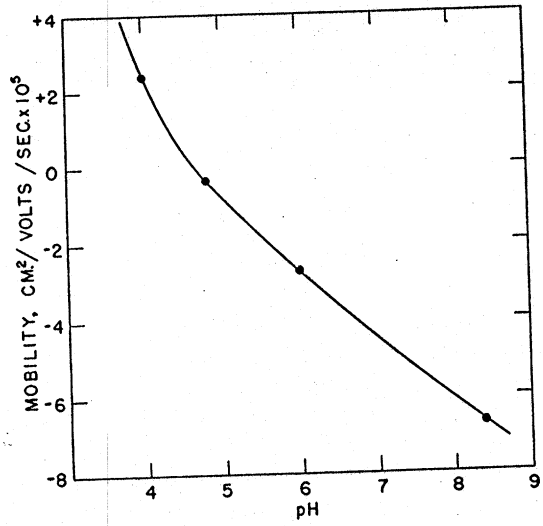

FIG. 2. Effect of pH on electrophoretic mobility of crystalline albumin of bovine whey in acetate and veronal buffers of 0.1 ionic strength.

with ammonium sulfate and treated as described in Step 3, yielding a crystalline albumin (500 mg.).

Step 1, *a* is modified by precipitating the 3.4 M ammonium sulfate fraction at pH 8.6. The filtrate is brought to pH 3 with 20 per cent trichloroacetic acid at 3°. The precipitate is allowed to settle overnight, the supernatant is decanted, and the remaining protein is removed by filtration. The paper containing the precipitate is macerated in dilute alkali solution, and the dissolved protein is fractionated further according to Step 3 to give a crystalline albumin.

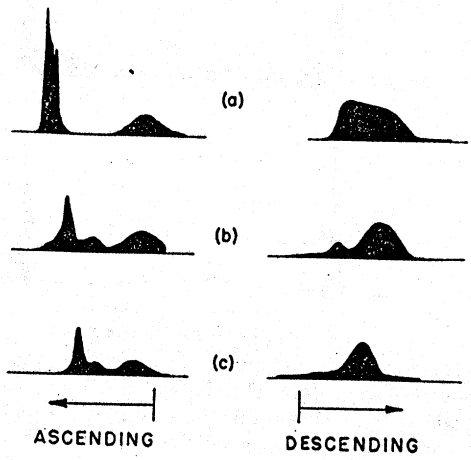

FIG. 3. Electrophoretic patterns of crystalline bovine serum albumin and whey albumin in acetate buffer at pH 4.0 after electrophoresis for 180 minutes. (*a*) Bovine serum albumin in buffer of 0.02 ionic strength, (*b*) crystalline whey albumin in buffer of 0.02 ionic strength, and (*c*) crystalline whey albumin in buffer of 0.1 ionic strength.

By these various procedures, from 0.5 to 1.0 gm. of crystalline whey albumin was obtained from 15 gallons of skim milk.

Properties

The crystalline whey albumin presented a single electrophoretic boundary in buffers of ionic strength 0.1 at pH values alkaline to the isoelectric point. In acetate buffers of ionic strength 0.1 or 0.02 at pH 4.0, the whey albumin showed a heterogeneous pattern similar to that described by Luetscher for serum albumin (8). The marked variations in components and mobility of crystalline bovine whey albumin as compared with serum albumin (Fig. 3) constituted the only electrophoretic differences observed between the two proteins. Mixtures of equal concentrations of the bovine whey and serum albumins migrated as a single component in veronal buffer at pH 8.4 and in acetate buffer at pH 4.8 with ionic strength 0.1.

The electrophoretic mobility of bovine serum albumin at 0° in veronal buffer of ionic strength 0.1 at pH 8.4 was -6.5×10^{-5} sq. cm. volt^{-1} sec.$^{-1}$. Under similar conditions, the mobility of crystalline whey albumin was -6.7. The electrophoretic mobility for serum albumin has been reported by Longsworth and Jacobsen (9) as -6.64 in veronal buffer at pH 8.56 and by Cohn *et al.* (6) as -6.0 in veronal buffer at pH 8.6.

Other physicochemical constants for the crystalline bovine whey and serum albumins are listed in Table I. These show agreement within the limits of experimental error.

TABLE I

Comparison of Physicochemical Properties of Crystalline Bovine Serum and Milk Albumin

	Crystalline albumin	
	Serum*	Milk
Per cent nitrogen†	16.0	16.1
Mol. wt.‡	69,000	69,000
Optical rotation $[\alpha]_{546}^{25}$§, *degrees*	-78 ± 2	-75
Isoelectric point‖	4.71	4.72
$E_{1\,cm.}^{1\%}$ at 280 mμ	6.6	6.6

* Constants for crystalline serum albumin were taken from the data reported by Cohn *et al.* (6) with the exception of the isoelectric point, which was reported by Longsworth and Jacobsen (9). The constants for the milk albumin were determined in this laboratory.

† Ash and moisture-free basis. Determined by Dr. C. L. Ogg.

‡ Determined by Halwer *et al.* by a light-scattering technique (10).

§ Measured in acetate buffer of ionic strength 0.1 at pH 4.8.

‖ The interpolated value for zero mobility obtained by electrophoresis of the albumin in acetate buffers of ionic strength 0.1.

Serological studies on the crystalline whey albumin by Dr. E. J. Coulson of the Allergen Division, United States Department of Agriculture, showed it to be identical with bovine serum albumin. These studies will be reported in detail by Dr. Coulson.

DISCUSSION

An electrophoretic study of the relation between the blood serum proteins and milk proteins in various stages of lactation has been reported by McMeekin *et al.* (11). The isolation of a crystalline albumin from milk with the properties of a crystalline serum albumin confirms and establishes the identity of the two proteins.

The use of small organic molecules as aids to crystallization with alcohol

has been amply discussed by Cohn *et al.* (6). The action, therefore, of caprylate as an adjunct for salt crystallization of bovine albumin is readily conceivable.

No explanation can be offered for the obvious difference in the electrophoretic patterns of the serum and whey albumins at pH 4.0. In the absence of specific experimental evidence, the argument that these variations reflect the interaction of the albumins with different anions or lipides can be considered only as a plausible and convenient hypothesis.

SUMMARY

It was possible to concentrate the albumin present in trace amounts in milk whey by salt and alcohol fractionation. Purified bovine milk albumin and serum albumin were crystallized with alkaline ammonium sulfate in the presence of phosphate and caprylate ions. Comparison of the physical, chemical, and serological properties of crystalline bovine milk albumin and serum albumin demonstrated the probable identity of these proteins.

BIBLIOGRAPHY

1. Bauer, J., and Engel, S., *Biochem. Z.*, **31**, 46 (1911).
2. Crowther, C., and Raistrick, H., *Biochem. J.*, **10**, 434 (1916).
3. Woodman, H. E., *Biochem. J.*, **15**, 187 (1921).
4. Wells, H. G., and Osborne, T. B., *J. Infect. Dis.*, **29**, 200 (1921).
5. Peskett, G. R., Annual Reports of the National Institute of Dairying, Reading, 52 (1932).
6. Cohn, E. J., Hughes, W. L., Jr., and Weare, J. H., *J. Am. Chem. Soc.*, **69**, 1753 (1947).
7. Palmer, A. H., *J. Biol. Chem.*, **104**, 359 (1934).
8. Luetscher, J. A., Jr., *J. Am. Chem. Soc.*, **61**, 2888 (1939).
9. Longsworth, L. G., and Jacobsen, C. F., *J. Phys. and Colloid Chem.*, **53**, 126 (1949).
0. Halwer, M., Nutting, G. C., and Brice, B. A., Abstracts, American Chemical Society, 116th meeting, Atlantic City, 3G (1949).
1. McMeekin, T. L., DellaMonica, E., and Custer, J. H., Abstracts, American Chemical Society, 112th meeting, New York, 27A (1947).